

## **IN THE SPECIFICATION**

*Please amend the specification at the paragraph beginning at page 2, line 24, as follows.*

In other ~~aspect~~, aspects, the invention relates to the use of valves with the plurality of actuators. For example, in one embodiment, a valve is coupled to a microfluidic network so that, when the valve is closed, it substantially isolates the second gas actuator from the first gas actuator. Such valves can control the direction of the propulsive force of the ~~actuators~~ actuators by preventing the expanding gas from traveling in certain directions, while permitting it to expand in the desired direction. They also extend the range over which an actuator can propel a microdroplet, by preventing the gas from dissipating in certain ~~[[in]]~~ areas upstream from the microdroplet.

*Please amend the specification at the paragraph beginning at page 5, line 10, as follows*

The chip carrier cartridge 120 is shown being inserted into (or removed from) an interface hardware receptacle of the DAQ 126 having electrical and optical contacts 125 standardized to mate with ~~[[a]]~~ corresponding contacts 121 of the chip carrier cartridge 120. Most contacts are for electrical signals, while certain ones are for optical signals (IR, visible, UV, etc.) in the case of optically monitored or optically excited microfluidic processors. Alternatively (not shown), the entire DAQ 126 may be a single ASIC chip that is incorporated into the Chip Carrier Cartridge 120, wherein contacts 121,125 would become conductive pathways on a printed circuit board.

*Please amend the specification at the paragraph beginning at page 6, line 27, as follows*

The oxide layer 140, heating elements 142, and resistive leads 144 are fabricated using well-known photolithographic techniques, such as those used to etch the microfluidic network.

*Please amend the specification at the paragraph beginning at page 7, line 22, as follows*

Because each actuator is preferably responsible for moving materials within only a subset of the modules of device 110, sample materials can be controlled more precisely than if a single actuator were responsible for moving material throughout the entire device. The various functional elements, of microfluidic device 110, including the actuators, are preferably under

computer control to allow automatic sample processing and analysis. Actuators 168, 170, 172 are connected to contacts 112.

*Please amend the specification at the paragraph beginning at page 8, line 11, as follows*

The multiple actuators and modules of microfluidic device 110 are preferably operatively connectable and isolatable by the valves of microfluidic device. For example, a closed state of either of valves 915, 216 operatively isolates microdroplet preparation module 170 from enrichment module 156. Thus, one or more actuators can be used to move materials between predetermined locations within microfluidic device 110, without perturbing or contacting material present in an operatively isolated module. The ability to operatively connect and isolate desired modules is advantageous in microfluidic devices having many process functions. Further, these valves also control the direction of the propulsive force of the ~~actuators~~ actuators by preventing the expanding gas from traveling in certain directions, while permitting it to expand in the desired direction. This also extends the range over which an actuator can propel a microdroplet, by preventing the gas from dissipating in certain ~~[[in]]~~ areas upstream from the microdroplet.

*Please amend the specification at the paragraph beginning at page 8, line 23, as follows*

The following demonstrates the cooperative operation of such multiple actuators in an example embodiment having a plurality of processing modules, namely an enrichment ~~zone 915~~, module 156, a microdroplet preparation module 158, a cell lysing module 160, a mixing module 166 and a DNA manipulation module 167.

*Please amend the specification at the paragraph beginning at page 9, line 5, as follows*

The enrichment module includes an enrichment ~~[[zone]]~~ chamber 931 (FIG. 5), a flow through member 900, valves 915, 919, and sample introduction channel 929. Valve 919 is connected between the flow through member 900 and actuator 168 as shown, and valve 915 is connected between the flow through member and a down stream channel 937 which leads to process module 158. These valves may be of any type suitable for use in a microfluidic device, such as thermally actuated valves, as discussed in co-pending application No. 09/953,921, filed

September 9, 2001. The valves may be reversible between the open and closed states to allow reuse of enrichment module 931.

*Please amend the specification at the paragraph beginning at page 9, line 17, as follows*

FIG. 5 is a cross-sectional view of the enrichment zone which shows the flow through member in greater detail. As shown, flow through member 900 has first and second surfaces 941, 943. First surface 941 is preferably adjacent enrichment chamber 931. Second surface ~~[[941]]~~ 943 is preferably spaced apart from the enrichment chamber 931 by flow through member 900. Flow through member 900 is preferably formed of a material having pathways smaller than the diameter of the particles to be enriched, such as pores of less than about 2 microns in diameter, for example, about 0.45 microns. Suitable materials for constructing flow through member 900 include, for example, filter media such as paper or textiles, polymers having a network of pathways, and glassy materials, such as glass frits.

*Please amend the specification at the paragraph beginning at page 10, line 20, as follows*

To enrich a sample, the device 901 operates as follows. ~~Referring~~ Referring to FIG. 4, valves 915, 919 are initially closed, and valve 913 is open. A particle-containing fluid is introduced into input port 180. Since valve 913 is open, it allows the sample to pass along channel 929 into enrichment zone 931. Alternatively, enrichment zone 931 can be configured to receive samples directly, such as by injection. Since valves 915 and 919 are closed, fluid is substantially prevented from escaping into actuator 977 and downstream channel 937.

*Please amend the specification at the paragraph beginning at page 12, line 30, as follows*

Microdroplet 802 is preferably defined by upstream and downstream boundaries each formed by a respective gas liquid interface 804, 806. The liquid of the interface is formed by a surface of a liquid forming the microdroplet. The gas of the interface is ~~[[gas]]~~ present in the ~~channels~~ microfluidic channels of microfluidic device 901.

*Please amend the specification at the paragraph beginning at page 13, line 12, as follows*

As explained above, actuator 168 of the ~~enriched~~ enrichment zone pushes the enriched sample into the microdroplet preparation zone 800. The enriched sample moves until reaching positioning element 979. In general, a positioning element inhibits the downstream progress of a microfluidic sample to thereby position the sample at a desired location. However, as explained more fully below, the positioning element does not permanently inhibit progress of the sample. Rather, it allows the microfluidic sample to continue downstream at a predetermined later time.

*Please amend the specification at the paragraph beginning at page 13, line 23, as follows*

Referring to FIGS. 8a-8b, gas actuator 170 is actuated, such as by DAQ 126, to thereby generate a gas pressure sufficient to separate microdroplet 802 from the second portion 822 of microfluidic sample 808. The gas pressure is preferably provided by the actuation of a heat source 958, which heats a volume of gas associated with gas actuator ~~[[957.]]~~ 170. As the pressure increases, the gas expands, thereby separating a microdroplet 802 from the rest of sample 808. Microdroplet 802 may comprise only a portion, such as less than about 75%, or less than about 50%, of microfluidic sample 808 received by microdroplet preparation zone 800. The dimensions of microdroplet 802 are determined by the volume of the channel between fluid barrier 979 and opening 820. For example, for a channel having a uniform cross-sectional area, a length  $l_1$  of microdroplet 802 corresponds to a distance  $d_4$  between positioning element 979 and opening 820. Thus, a microfluidic device can be configured to prepare microdroplets of any volume by varying the length between the fluid barrier and corresponding actuator opening.

*Please amend the specification at the paragraph beginning at page 14, line 13, as follows*

As shown in FIGS. ~~4 and 12~~, 4, 12a and 12b, lysing module 160 includes a lysing zone 950, a lysing mechanism within the lysing zone (such as electrodes 954), and a vented positioning element 200 positioned upstream from the lysing zone. The lysing mechanism preferably includes a set of electrodes or other structures for generating electric fields within the lysing zone. The vented positioning element preferably includes a vent 202, a valve 204, and a second positioning element 206 for inhibiting fluid from flowing into the vent.

*Please amend the specification at the paragraph beginning at page 16, line 1, as follows*

In an alternative embodiment, a lysing module 300, as shown in FIGS. 13a, 13b, includes a lysing zone 302 which is configured to prepare a lysed microdroplet 304 of predetermined volume from a microfluidic sample 306, which may have an indeterminate volume. Lysing zone 302 preferably includes a lysing mechanism such as electrodes 308. Electrical leads 310 provide a connection to a pulse circuit of DAQ 126, via contacts 112, between microfluidic cartridge 110 and chip carrier 120, and contacts 125 (as shown in FIGs. 1 and 3). A positioning element 312 is disposed downstream of lysing zone 302. An actuator 314 is disposed upstream from lysing zone. Actuator 314 preferably includes a second positioning element 316 to prevent fluid from the microfluidic sample from entering therein.

*Please amend the specification at the paragraph beginning at page 19, line 21, as follows*

A positioning element 979 may be formed by a non-wetting material disposed to contact a microfluidic sample. The ~~physio-chemical~~ physico-chemical properties of the non-wetting material are chosen upon considering the type of liquid forming the microfluidic sample. For example, where the microfluidic sample is an aqueous sample, the positioning element preferably comprises a hydrophobic material. An exemplary hydrophobic material includes a non-polar organic compound, such as an aliphatic silane, which can be formed by modifying an internal surface of microfluidic device 901. For microfluidic samples formed of organic solvents, the non-wetting material may comprise a hydrophilic material.

*Please amend the specification at the paragraph beginning at page 20, line 6, as follows*

Referring to FIGS. 10a-10c, another type of positioning element may be formed by modifying the dimensions of the microfluidic channel to form a capillary assisted positioning element (CAFB) 700. A CAFB comprises an upstream feed zone 702, a loading zone 704, and a stop zone ~~[[704.]] 706~~. A microfluidic sample 720 encountering the CAFB moves downstream until a downstream interface 710 of the microfluidic sample contacts upstream surfaces ~~[[714]] 712~~ of the loading zone ~~[[706.]] 704~~. At this point, capillary action causes the microfluidic sample to move downstream until the downstream sample interface 710 encounters the opening ~~[[712]] 714~~

between the loading zone 704 and the stop zone 706. Surface tension resists the tendency of the microfluidic sample to continue downstream past opening 714. Thus, the microfluidic sample 720 is positioned at a predetermined location along the channel axis with respect to positioning element 700.

*Please amend the specification at the paragraph beginning at page 20, line 17, as follows*

The volume of the microfluidic sample encountering the CAFB preferably has a larger volume than a volume of the loading zone 704 to ensure that the microfluidic sample will advance fully to opening. For fluids that have similar surface tensions and interface properties as water, the depth  $d_1$  of the loading zone 704 is preferably about 50% or less of the respective depths  $d_2$ ,  $d_3$  of the ~~[[stop]]~~ feed and ~~[[feed]]~~ stop zones.

*Please amend the specification at the paragraph beginning at page 21, line 14, as follows*

An open state of a valve 512 allows passage of gas between zone 510 and vent 508. A closed state of valve 512 prevents such passage of gas. Valve ~~[[514]]~~ 512 is preferably thermally actuated and includes a mass 514 of ~~[[TRS.]]~~ thermally responsive substance (TRS).

*Please amend the specification at the paragraph beginning at page 21, line 17, as follows*

Positioning element 500 preferably operates as follows. Referring to FIG. 11a, microfluidic sample 502 moves downstream in the direction of arrow 524. Microfluidic sample is preferably moved by a gas pressure provided from an upstream actuator, which is not shown in FIGS. ~~9a-9c~~ 11a-11c. The gas pressure acts upon upstream portion 504.

*Please amend the specification at the paragraph beginning at page 22, line 25, as follows*

Upon recognition that the ~~liquid~~ liquid has arrived at sensor 672, the DAQ instructs second actuator 660 to decrease a downstream motive force acting upon the microfluidic sample 666. For example, DAQ may reduce a current flowing through a heat source 676 associated with second actuator 660 thereby reducing a temperature of a gas therein. The temperature reduction reduces the gas pressure acting upon a upstream portion 678 of microfluidic sample thereby inhibiting the downstream motion of the microfluidic sample 666. The microfluidic sample is

positioned such that a first portion 680 is located downstream of actuator 656 and a second portion 682 is located upstream of actuator 656.

*Please amend the specification at the paragraph beginning at page 23, line 3, as follows*

To prepare microdroplet 668, DAQ 126 actuates actuator 656 to provide a motive force which prepares the microdroplet 668 from the first portion 680 of microfluidic sample 666.

Microdroplet 668 moves downstream while the second portion 682 of the microfluidic sample 666 moves upstream from actuator 656. During microdroplet preparation, valve 658 may be closed to substantially isolate the actuator 656 from second actuator 660 and other upstream portions of the microfluidic device.